

leached, with a consequence that the soils have low available P levels due to Al fixation of P. Under these conditions VA mycorrhizae may be important in the rapid cycling and uptake of inorganic P.

Ectomycorrhizal Fungal Fruiting Bodies as Nontimber Forest Products

Fruiting bodies of ectomycorrhizae are commonly collected and sold. In some forest areas the value of the mycorrhizal fungal fruiting body harvest exceeds the value of wood production. Harvesting is mainly restricted to a small number of valuable species such as *Boletus edulis*, *Cantharellus cibarius* (chanterelles), *Tuber magnatum* (white truffles), and *T. melanosporum* (black truffles).

See also: **Health and Protection:** Biochemical and Physiological Aspects. **Tree Physiology:** A Whole Tree Perspective; Nutritional Physiology of Trees; Root System Physiology.

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Physiology of Sexual Reproduction in Trees

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Introduction

The physiology of sexual reproduction in forest trees has progressed substantially over the past 20 years. Three previous symposia of the International Union of Forestry Research Organizations (IUFRO) not only summarized current knowledge but also laid out

the ground work for future studies. Many of these questions are still valid today but substantial progress has been made in early maturation, flower induction, and pollination biology. Many questions still exist (mechanism of flowering) and new questions are being asked (environmental effects on progeny performance).

New technology, principally molecular techniques, is now available and offers exciting new possibilities for future flowering, pollination, and seed production studies. However, there has been a substantial shift in flowering research. Considerably fewer studies are being published on the mechanism of flowering in conifers but, equally so, the volume of work dealing with hardwoods, especially tropical hardwoods, is growing.

The perspective of this contribution in forest tree seed production has a definite commercial bias. Procuring seed as a mechanism for delivery of tree improvement programs as well as plantation forestry has been the incentive for pursuing both fundamental research and program development. Much of the emphasis has been with seed orchard development simply because it is the most cost-effective tool for delivery of genetic gain.

Developmental biologists may object to my use of the word 'flowering' when referring to conifers. Certainly the use of 'sporangiate strobili' would be more precise but also more awkward. Here 'flowering' is restricted to the general process of sexual reproduction in conifers. The terms 'seed' and 'pollen cones' refer to the mega- and microsporangiate cones, respectively.

Plantation Forestry

According to statistics provided by the Food and Agriculture Organization, global forest plantations have grown from about 18 million hectares in 1980 to 187 million hectares in 2000 (both hardwoods and conifers). This represents about 5% of the total global forest cover. Asia accounts for about 62% of the current plantation area, followed by Europe (17%), North and Central America (9%), South America (6%), Africa (4%), and Oceania (2%). Globally, broadleaved trees make up 40% of the total, of which *Eucalyptus* is the principal genus and conifers make up 31%, of which *Pinus* is the principal genus. Over the past decade, the percentage of these plantations destined for industrial use has increased from 36% to 48%. Tree improvement, as part of plantation forestry, has been a principal driver of flowering research, especially for temperate conifers.

Critical to the success of these programs is a sustainable supply of not only the species but also

adapted families/provenances for the designated target areas. Propagules from asexual techniques (rooted cuttings and somatic embryogenesis) will likely increase but for the foreseeable future seed production will supply the bulk of our needs. The single most important source of seed will continue to be natural stands but in many countries, tree improvement and seed orchard programs are beginning to make substantial contributions of seed. In Canada, an estimated 3.9 billion seeds were sown in 1999, of which 0.63 billion (16%) came from seed orchards. The proportion of seed from seed orchards in Canada is expected to rise to about 80% in the next decade.

Seed orchards offer substantial advantages over natural stand production. With the correct choice of sites, flowering is more regular and inducible. Seed yields are higher because pollen supply is not limiting and crop protection measures (insect control) are more readily applied. When seed orchards are based on selected material from genetic improvement programs, the opportunity to take advantage of family-specific traits is available. This will become very important in the near future if forests cannot adapt to global warming. Tropical species will be less affected but north temperate species may not realize predicted gains and, at worst, plantations may fail. In order to respond to these new demands, we must know the reproductive biology and genecology of our species, including adaptive traits of families as well as the effect of environment on reproductive success and progeny performance. This is the challenge for future physiologists.

Reproductive Biology

The reproductive biology of forest trees forms the basis of our current and future studies. The reproductive biology of species includes the cycle of events starting from initiation of reproductive structures through to seed dispersal. For most conifers and temperate hardwoods these cycles have been at least partially, if not fully, described. In general, this information is lacking for tropical hardwoods but we expect to see this knowledge base expand rapidly over the next few years. Before a tree begins its reproductive cycle, it must first become sexually mature.

Sexual Maturation

All trees go through a phase of juvenility before flowering occurs and this period can be short (1–2 years) or long (greater than 30 years). There are specific phase changes associated with maturation in woody plants. These include morphological changes

(decrease in growth rates, an increase in plagiotropism, a change in foliar morphology, and the onset of reproductive competence) as well as physiological and biochemical changes. While maturation is likely under genetic control, the effect of environment on gene expression is also significant. For example, many species of the family Pinaceae and Cupressaceae can become sexually competent in just a few years from seed. Using a combination of early growth acceleration (extended photoperiod and optimal growing regimes), applied stress (drought and heat during reproductive differentiation), crown-forming and growth hormones (gibberellins), flowering can be induced in just a few years and as low as 1 year. Sexual expression is a result of not only the attainment of ripeness to flower (maturation) but also the exposure to environmental/cultural conditions that stimulate flowering.

Physiology of Flowering

The anatomical and morphological description of flowering in conifers and temperate hardwoods has been thoroughly detailed. There are several critical stages in the reproductive cycle to know and they are most often referenced to calendar date. However, phenological development is mediated by environmental cues (photoperiod, temperature, soil moisture), making calendar dates too variable. Where possible, reproductive stages should be referenced to a readily observable phenological stage of development (i.e., bud burst and bud set) of the shoot or plant.

The reproductive stages of most interest are floral initiation and differentiation, meiosis, anthesis, fertilization, embryo development, and cone maturation. All reproductive structures of conifers and hardwoods differentiate from undetermined terminal or axillary apices. For all species of Pinaceae and Cupressaceae, initiation and differentiation are completed before winter dormancy, with the possible exception of soft (Haploxylon) pines where anatomical identification of seed cone structures does not appear until the spring following dormancy. However, in these species, the biochemical/physiological events leading up to differentiation and anatomical identification may occur prior to winter dormancy.

Conifer apices can abort, remain latent, or be determined as seed cones, vegetative buds, or pollen cones. The development sequence apices undergo is largely determined by their position on the stem and in the crown as well as the physiological state of the tree. Temperature and soil moisture conditions that put the trees into stress during periods of initiation and differentiation are the principal

environmental factors determining the fate of apices. Light has also been implicated as a contributing factor but, aside from regulating shoot growth, light does not appear to play a direct role in the flowering of conifers. For tropical species, light (photoperiod) and temperature are important factors affecting flowering. Soil nutrient status (fertilizer) also affects flowering but the results are often conflicting and seldom predictable.

Mechanism of Flowering

We do not know the specific mechanism of flowering in trees nor why trees respond to specific inductive treatments. We do know, however, many of the environmental triggers that induce flowering in most Pinaceae and Cupressaceae species and some tropical hardwoods but we have yet to explain the mode of action of specific induction treatments acting either alone or in combination. Much of what we know today about flower induction in temperate conifers came from observations made in natural stands. Trees growing in open, more exposed sites on drought-prone soils are more conducive to flowering than those growing on sites that are shaded, cool, and moist. While some stress is essential for flowering to occur, it is stress applied at specific stages of phenological development that is important. Both low annual rainfall and above normal temperatures in the preceding year are associated with good flowering. However, we do not know how much (or little) drought and heat is required, although experience suggests that warm springs followed by warm, dry summers in temperate climates are best. We also know that growth in the years preceding flowering is important. Even if optimal meteorological conditions leading to stress differentiation occurs, flowering may not necessarily occur. Flowering the year following a particularly heavy crop year (mast) rarely occurs. For species that form their seed cones in the terminal position (i.e., spruce), then new growth must occur before another flowering event can occur.

Several hypotheses have been proposed to explain flowering in conifers. All implicate flowering in relation to shoot growth and plant hormone metabolism. A nutrient-diversion hypothesis contends that a higher concentration of nutrients is required for buds to differentiate reproductively and treatments which retard shoot growth will favor flowering. A second hypothesis relates bud (meristematic) vigor to flowering. Very-high- or very-low-vigor buds remain vegetative. Buds of moderate activity would preferentially promote female flowering in the upper crown (higher-vigor shoots) and male flowering in the lower crown (lower-vigor shoots). The effect of

cultural stress treatments (root-pruning, root restriction, drought, and girdling) would be to slow the growth of vegetative buds and favor (enhance) the development of reproductive buds.

However, these hypotheses do not explain the results of gibberellin A_{4/7} (GA_{4/7}) applications which not only promote flowering but can also enhance shoot growth (with no effect on apical bud activity). This suggests that the hormonal promotion of flowering and shoot growth are independent processes. Cultural treatments, such as root-pruning (in Douglas-fir), which also enhance flowering, did so by delaying development and not by increasing nutrient concentrations (total insoluble carbohydrate) in axillary apices. Root-pruning also reduces cell elongation and slows apical bud (mitotic) activity in both lateral and terminal buds.

A plausible hypothesis exists for the enhancement of flowering in conifers based on gibberellin biosynthesis and metabolism, although it remains largely untested. The effect of cultural treatment with and without GA_{4/7} treatments suggests that flowering is under numerous biochemical/physiological controls, all of which must be 'permissive' if reproductive structures are to be differentiated and developed. Furthermore, cultural treatments (root pruning, drought, girdling, fertilizer, high temperature, root flooding) may all act through effects on GA metabolism. The effect of these six cultural treatments seems to increase the less polar endogenous GA-like substances (GA_{4/7}, GA₉) while at the same time decreasing the levels of more polar-like GA substances (GA₃). Results from labeled (tritiated and deuterated) GA₄ studies support this hypothesis and further suggest that GA activity is regulated in the shoot cell and their metabolites may be influenced by factors affecting root activity.

Roots may have a direct role in regulating flowering and it may involve cytokinins. The promotion of flowering by GA_{4/7} alone and in combination with root flooding resulted in a marked decrease in cytokinin levels in Douglas-fir shoots harvested during the period of cone-bud differentiation. This led to the suggestion that actively growing roots export substances inhibitory to flowering. Since cytokinins are known to inhibit GA biosynthesis, the decreased levels of cytokinins found during flower promotion treatments may actually raise the levels of less polar GAs and, conversely, the higher levels of root-exported cytokinins during nonstress periods would result in lower GA levels and no flowering response.

While this hypothesis is intriguing, it must still be confirmed for the many species in which flowering has been promoted through application of stress

treatments. While we do not have hard evidence that less polar GAs have a direct morphogenic role in the promotion of flowering in conifers, it is currently the best one we have. With the increasing knowledge of physiological processes under genetic control and the rapid expansion of molecular technique, new and innovative techniques can now be applied to test this hypothesis rigorously.

It is also interesting to note that flowering in the Pinaceae species only responds to the less polar gibberellins, GA_{4/7}, and species of the Cupressaceae families only respond to a more polar gibberellin, GA₃. For Pinaceae species, the effect is enhanced if treatments are combined with stress-inducing cultural techniques such as drought, root-pruning, and heat. Adjunct stress treatments for Cupressaceae species are less effective. Gibberellins do not seem to affect a flowering response in hardwoods but they do respond to photoperiod and shoot-growth retardation which may result in a reduction of GA concentrations. Applications of the growth retardant paclobutrazol have successfully induced flowering in *Eucalyptus* and in a dipterocarp (*Shorea stenoptera*).

Sex Expression

Because most conifer species produce large quantities of pollen that can be handled relatively easily *ex situ*, developing techniques to increase the ratio of seed cones to pollen cones would facilitate not only improved seed production but also the delivery of genetic gain through seed orchard management.

The management of seed production has long sought after sex-specific flower induction treatments. Most conifer species are monoecious and pollen cones are normally differentiated before seed cones. This has led to the possibility that both specific induction treatments and their timing may favor the response of one sex over the other. Cultural treatments are less specific but GA application may provide our best opportunity to control sex expression. However, the levels of endogenous GA normally applied are so large relative to exogenous levels (estimated to be about 5000 greater), it is difficult to expect such subtle responses at the cellular level. Furthermore, we know very little about other plant hormones and their role in sex expression. Certainly, the balance of gibberellins, auxins, cytokinins, and possibly growth inhibitors (i.e., abscisic acid) has been implicated in other plants (angiosperms) and to a certain extent in conifers but their mode of action in conifers is far from clear. Further, the interaction of known growth regulator response with cultural treatments and photoperiod must be very complex.

Regardless of these uncertainties, the value of sex-specific treatments remains. Current management practices that show promise are crown-pruning techniques that favor shoots with a high potential of pollen or seed cone response and treatment timing (specifically gibberellins) in relationship to shoot phenology. We also have the possibility of sex-specific gametocides, including chemical agents that inhibit pollen development.

Pollination Biology

Our knowledge of pollen biology and handling pollen *ex situ* has made pollen management of conifers an essential activity for breeding programs. Pollen management (supplemental pollination) can also be cost-effective in seed orchards where external pollen supply (contamination) can reduce the genetic worth of seed lots or in young orchards where pollen supply and distribution limit seed production. It can also be an effective tool for producing seed with specific traits (i.e., insect-resistant progeny).

Further restraints to seed production and optimal genetic gain include differential parental contribution and nonrandom mating. Some parents are more fecund than others and nonsynchronous flowering (seed cone receptivity and pollen shed) produce distinct breeding populations within orchard seed lots. Orchard activities to balance parental contribution and random mating among all parents include flower enhancement of selective clones, orchard cooling to reduce synchrony between external orchard pollen flight and within orchard pollen flight, and pollen management.

Pollen management involves the collection, extraction, storage, testing, and reapplication of pollen. To handle pollen successfully, we must be able to maintain and measure pollen viability *ex situ*. Pollen of all Pinaceae species is relatively easy to collect, extract, and store. Optimal storage conditions vary slightly among species. In general, pollen must be harvested as near to natural shedding as possible, dried at low humidity (30–40%) and warm temperatures (25–30°C) to a moisture content between 5 and 10%, and then stored in airtight containers at freezer temperatures (–30°C or lower). For the few Cupressaceae species tested (i.e., *Thuja plicata*, *Chamaecyparis nootkatensis*), pollen must be handled differently. First, pollen must be extracted under high humidity (70–80% relative humidity) and cool temperatures (15–18°C). Under these conditions, pollen moisture content ranges from about 15% to 20% and pollen viability can be maintained at freezer temperatures.

However, conifer pollen quality can deteriorate quickly, especially under high temperatures and humidity. If pollen is exposed to a wide range of conditions, then it is important to test its *in vitro* viability before using. For conifers, three assays that provide consistently good estimators of field performance are respiration (a measure of oxygen uptake), electrical conductivity (a measure of membrane stability), and germination (pollen tube growth in a nutrient medium). Respiration and conductivity are straightforward assay procedures and yield the most consistent response for a wide number of species tested (Pinaceae and Cupressaceae). Germination can also yield good results but the procedure is very sensitive to assay conditions, in particular cultural technique, media components, and pollen prehydration.

Estimates of a pollen lot's fertility potential (ability to set seed) can be determined from assay response. Seed set rises rapidly with increasing pollen viability but quickly reaches an asymptote determined by the number of viable ovules per cone. Since most studies relating assay response to seed set are done using controlled crossing (isolation bags) procedures, the interpretation of these results is limited to this particular pollination technique. When using viability assay response for pollen lots to be used in pollen mixes (i.e., supplemental pollination), then competition between pollen lots becomes important. Since we expect equal contribution from each pollen lot within a supplemental pollination mix, variation in viability may lead to disproportionate contribution. Now that molecular fingerprinting techniques are routinely available, it is possible to measure paternal contributions and use this information to formulate mixes (equal volumes or adjusted to viability response) and to relate *in vitro* viability response to competition both within supplemental pollination mixes and between endogenous orchard pollen clouds.

Pollination Mechanism

Conifers and most temperate hardwoods are wind-pollinated (anemophilous) whereas most angiosperms are insect-pollinated. Most conifer pollen is shed by a wind shock and then air flow patterns around the receptive seed-cone bud not only aid the probability of pollen entrapment but may also favor pollination from its own species. While pollen capture is not specific (it requires over 1 million per ovule), the mechanism of pollen capture can be very efficient.

Once captured, the mechanism of moving the pollen grain into micropyle is quite different among conifer species. A pollination drop is actively involved in some species but not in others. There is considerable interest and, to a large extent, uncer-

tainty about the actual role of the pollination drop. It has not been detected in species such as *Pseudotsuga*, *Larix*, *Tsuga*, and *Abies*. Where the drop does exist (i.e., *Pinus*, *Picea*, Podocarpaceae) and the micropylar arms of the ovule are inverted, the pollination drop can actively draw the pollen grain into the micropyle as it recedes. In some species, such as *Thuja* and *Chamaecyparis*, the pollination drop simply acts as a medium for the pollen landing on its surface to sink towards the nucellus. The various types of pollination mechanism are based on the presence or absence of a pollination drop and may have evolutionary significance.

The pollination drop may be a more recent occurrence but others argue that ovule secretions must have been part of the primitive pollination mechanism as well. Regardless of its evolutionary significance, conifer pollinations drops play an important role in not only directing the pollen towards the nucellus but also in preparing the grain for germination and, perhaps, even actively selecting the species' own pollen from foreign pollen and other microbes. The constituents of the drop include many simple sugars, amino acids, and proteins. With improving analytical technique, the complexities of ovule secretions and the role they may play in pollen germination, selfing and male selection will become clearer.

Cone Development

The description of conifer cone development from preanthesis to seed formation has been extensively reviewed. However, the effect of cultural and environmental conditions on these processes has been less studied, in particular seed cone development. Most temperate Pinaceae species develop their reproductive structures early in the season when temperatures are cool and soil moisture is adequate. However, development of ovules, pollen uptake, and fertilization may be affected by seed orchard environments prone to drought and high temperatures. Most often, the principal cause of poor seed set in conifers is unpollinated ovules. Even when artificial pollen is applied, on average seed yields do not normally exceed 50–60% potential yields. There are also a considerable number of immature or poorly developed ovules in each cone, typically at the base and tips of the cone. Certainly, cone development and early ovule development are under a certain degree of genetic control but the potential to increase the number of mature ovules through cultural techniques warrants a closer look. This is especially true in seed orchard environments where culture techniques can be easily applied.

Another factor affecting low seed set is cone and embryo abortion. *Pinus* species are most sensitive to pollen supply where a substantial percentage of first-year conelets can abort due to inadequate pollen. However, in mature seed orchards, pollen supply is seldom limiting and cone retention is high. However, under field conditions where drought, high temperatures, and low humidity occur, poor uptake of pollen in *Pinus* species may result in increased cone abortion. It is also possible that drought, high temperatures, and low humidity could restrict fertilization and early embryo development in all species.

Environmental Effects on Reproduction

Orchard environment can affect progeny performance. Progeny from parent trees growing in warmer southern seed orchard environments can display delayed flushing in the spring and extended growing periods and delayed growth cessation in the summer. They also developed frost-hardiness later compared to like genotypes derived from northern sources. This phenomenon has been termed seed orchard aftereffects and the effects can endure for several years, if not the lifetime of the tree. Similar effects have been demonstrated with progeny derived from like parents growing under greenhouse conditions compared to open orchard conditions. Further, progeny derived from seed reared under the warmer environment of greenhouses can also show lower spring and fall frost resistance.

We do not know the mechanism of this phenomenon, nor do we know at which stage of reproductive development the effect occurs. However, several hypotheses have been postulated. Since many of the genes expressed in the sporophyte are also expressed in the gametes, it is possible that gametophytic selection occurs. It is argued that pollination selection does not occur in conifers because the pollination mechanism allows too few pollen grains to enter the micropyle to provide sufficient selection pressure. However, three or four pollen grains in each of 100 ovules per cone and hundreds of cones per tree may provide a population sufficiently large to consider a directional selection of pollen under different temperature regimes. Furthermore, conifers have multiple archegonia (many egg cells) per ovule, which permits the possibility of temperature affecting the selection of many fertilized egg cells. Finally, the effect could also be non-Mendelian and represent a maternal effect that remains to be described.

See also: **Ecology:** Plant-Animal Interactions in Forest Ecosystems; Reproductive Ecology of Forest Trees. **Tree Physiology:** Physiology of Vegetative Reproduction;

Shoot Growth and Canopy Development; Tropical Tree Seed Physiology.

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Forests, Tree Physiology and Climate

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Introduction

Climate can be defined as the long-term integration of atmospheric parameters such as temperature, precipitation, wind, insulation, air humidity, or snow cover. It is one of the key factors conditioning the growth of vegetation and it is impossible to dissociate